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16. ABSTRACT

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1 mg O2/l
(2.5 mg/l
(1 mg O2/l
2.5 mg/l
1 mg O2/l

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Oxygen requirements of larvae of the smallmouth bass, *Micropterus dolomieui* Lacépède

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Smallmouth bass larvae became highly sensitive to oxygen deficiency on the second day after hatching and continued so to the 10th day. During this period they could not survive exposure to 1 mg O_2 l^{-1} for 3 h at 20° C, and many were killed within 1 h. At 2 mg O_2 l^{-1} half the larvae survived 3 h at 20° C; at 2·5 mg l^{-1} most survived, and at 3·5 mg l^{-1} all survived. Resistance to oxygen deficiency was regained by the 11th day, the majority of the larvae withstanding a 3-h exposure at 1 mg O_2 l^{-1}. At 25° C the effects of low oxygen concentration were intensified. At 3 and 4 mg O_2 l^{-1} and 20° C the normally quiescent larvae became very active, even swimming to the surface 5 or 6 cm above the substrate. Increasing the temperature increased this response. Smallmouth larvae were more sensitive than largemouth bass larvae to oxygen deficiency.

I. INTRODUCTION

Despite the similarities between adults of smallmouth bass *Micropterus dolomieui*, and largemouth bass, *Micropterus salmoides*, smallmouth larvae are twice the size of largemouth at hatching, and they remain on the substrate twice as long (Scott & Crossman, 1973). It has been shown (Spoor, 1977) that largemouth larvae are especially sensitive to oxygen deficiency on the third day after hatching, but more resistant before and after that day. Lowering the oxygen concentration to 3 or 4 mg l^{-1}, which is above the lethal range, at any time during the larval period causes them to become highly agitated, and many swim up from the substrate so far that under natural conditions they could be lost from the nest. The present study was undertaken to determine whether the greater size and slower development of smallmouth bass larvae make their oxygen requirements different from those of the largemouth. That these requirements might be different is suggested by the poorer survival of smallmouth embryos and larvae maintained at an oxygen concentration of half saturation (Carlson & Siefert, 1974; Siefert *et al.*, 1974).

II. MATERIALS AND METHODS

The methods were the same as those described previously by Spoor (1977). The larvae were tested in screenbottomed trays (15 × 3 × 7 cm high) through which water of controlled dissolved oxygen content flowed continuously at 50–60 ml min^{-1}. Except during a few tests of the effect of temperature on oxygen requirement, the larvae were kept at 20 ± 0·2° C. A 16:8 h LD photoperiod was maintained, and dawn and dusk were simulated (Drummond & Dawson, 1970). The light intensity by day varied from about 25 Lm.

The water was drawn directly from Lake Superior; the pH was 7·6–7·8 and the major inorganic constituents (approximate mean values in mg l^{-1}) included Ca, 14; Mg, 3; Na,

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1·1; K, 0·5; Cl, 1·2; SO_4, 3·2; and HCO_3, 40. Some of the water was aerated vigorously to approximate gas equilibrium with the atmosphere, and some was passed through a vacuum degassing apparatus (Mount, 1964) to reduce its oxygen content to less than 1 mg l^{-1}. Water of the desired oxygen content was obtained by mixing aerated and de-aerated water; an oxygen electrode was used for the adjustment. All oxygen concentrations were confirmed by the unmodified Winkler method.

Embryos and larvae were obtained from the field. Three sets of embryos were taken from nests in hatchery ponds at the National Fish Hatchery at New London, Minnesota, one set in each of the years 1975, 1977 and 1979. These embryos were brought to 20° C upon arrival at the laboratory, where they hatched within 2 days. Two sets of larvae were taken from nests in Two Island Lake, near Grand Marais, Minnesota, in 1977. The temperature was about 20° C in the vicinity of the nests, and the larvae were maintained at that temperature in the laboratory. Specimens from each of the five groups, representative of each day of development and of the effects of treatment, were examined fresh and after preservation in 4% formaldehyde. The ages of the larvae from Two Island Lake were estimated by comparing them with the developmental stages of New London larvae of known hatching date. It was concluded that one group was collected on the first day after hatching, the other on the fifth day.

Although their origins and years of collection were different, the five groups of larvae had essentially the same developmental histories. At 20° C and 8 or 9 mg O_2 l^{-1} most larvae remained on the substrate until the ninth day after hatching. Some started swimming up for short periods on the seventh day, more did so on the eighth, and continuous mid-water swimming started on the ninth day, by which time most of the yolk had been consumed. From the 10th day all were swimming and taking brine shrimp nauplii, as reported by Siefert *et al.* (1974).

For survival tests, groups of 10 or more larvae, depending upon availability, were transferred to the screened trays with a wide-bore pipet and exposed to a reduced oxygen concentration of 1, 2, 2·5 or 3·5 mg l^{-1} for a 1, 3, or 6 h period on 1 day during development, beginning with the first day after hatching and ending with the 12th. Before and after the exposure the test groups were maintained at 20 ± 0·2° C and about 9 mg O_2 l^{-1}; the controls were kept under these conditions throughout. Most tests were conducted at 20 ± 0·2° C, but a few were conducted at 25 ± 0·2° C.

Oxygen consumption was measured in continuous-flow respirometers similar to the screen-bottomed trays but isolated from the air (Spoor, 1977). Groups of 30 to 100 larvae were maintained at 20 ± 0·2° C in the respirometers until after they had begun to feed. Their oxygen consumption was measured daily. The oxygen content of the effluents from experimental and blank chambers as measured with an oxygen electrode or by the Winkler method with 0·01 N titrant. The water flow-rate was reduced to between 20 and 40 ml min^{-1} to increase the difference between experimental and blank readings, but the oxygen uptake was so small, usually with the range 0·1 to 0·4 mg l^{-1}, that the measurements varied considerably.

For studying behaviour at different oxygen concentrations, 10 1-day-old larvae were placed into each of three screen-bottomed trays with continuously flowing 20° water. Each tray was arranged so that water of different oxygen concentration, and occasionally of lower temperature, could be introduced without disturbing the fish. Behavioural responses were noted daily for 12 days.

III. RESULTS AND DISCUSSION

AGE AND SENSITIVITY TO OXYGEN DEFICIENCY

Like the larval histories, the results of the survival tests were similar among the five groups. Because the numbers of specimens were limited, the results have been combined.

Before hatching and on the first day thereafter the embryos and larvae were highly resistant to oxygen deficiency: 90% or more survived 6 h at 1 mg O_2 l^{-1} and 20° C. Starting with the second day the larvae became increasingly sensitive, and

TABLE I. Growth and oxygen consumption of smallmouth bass larvae at 8–9 mg O_2 l^{-1}, and survival for 1 or 3 h at 1±0·1 mg O_2 l^{-1}, at 20±0·2° C on days 1 through 12 after hatching

Day after hatch	Mean yolk-free weight (mg)*	Oxygen consumption (μg larva^{-1} min^{-1})†		Survival (%) at 1±0·1 mg O_2 l^{-1}‡	
		mean	range	1 h	3 h
1	2·25	—	—	95	90
2	2·67	0·040	0·032–0·050	84	40
3	3·21	0·038	0·032–0·049	90	10
4	4·09	0·056	0·044–0·071	95	0
5	4·18	0·059	0·048–0·068	62	0
6	4·24	0·066	0·055–0·081	32	0
7	6·18	0·088	0·066–0·116	32	0
8	7·31	0·107	0·087–0·132	30	3
9§	8·42	0·133	0·098–0·165	14	0
10§	9·35	0·133	0·098–0·180	48	0
11¶	9·73	0·117	0·095–0·137	40	70
12¶	9·46	0·121	0·086–0·145	61	60

*With the exception of day 1, for which there were but four specimens, each weight is the mean of 10–20 specimens preserved in formaldehyde, dissected free of yolk, and drained of excess water on filter paper.

†Means and ranges; each value based on 200–400 larvae, data from three to five groups combined.

‡Each value based on at least 10 larvae; usually 20–40.

§Swim-up and feeding started.

¶Swim bladder becoming inflated.

by the fourth day none survived 3 h at 1 mg O_2 l^{-1} (Table I). This sensitivity continued to the 10th day, but by the 11th and subsequently they were able to survive the exposure. Judging by the resistance to 1 h exposure, days 6 to 9 were the most critical. As noted with largemouth bass larvae (Spoor, 1977), some of the smallmouth larvae remaining alive after a test had the lower jaw locked in a fully opened position. Because these larvae did not recover, did not swim up, and faced starvation because they could not feed even after the 12th day, they were counted as dead.

An increasing oxygen requirement seems a probable cause of the increasing sensitivity: the mean oxygen consumption increased from about 0·04 μg larva^{-1} min^{-1} on day 2 after hatching to about 0·13 on day 9 (Table I).

Because the onset of opercular movements seemed to improve the ability of largemouth bass larvae to survive reduced oxygen concentrations (Spoor, 1977), the development of the opercula and gills was observed on living smallmouth larvae each day between hatching and swim-up. Here the smallmouth bass differed from the largemouth. Rhythmic opercular movements began on the fourth day, which was the first day on which none could survive 3 h at 1 mg O_2 l^{-1}, and blood could be seen flowing through the gill arches by the sixth day. Since the larvae did not recover resistance to oxygen deficiency until the 11th day, something more than these developments was required.

It is possible that the development of the swim bladder contributed to recovery.

TABLE II. Numbers of smallmouth bass larvae dead, alive but with jaw locked, or surviving with no apparent injury after exposure to low oxygen concentrations for 1, 3, or 6 h at 20° or 25° C

Dissolved oxygen (mg l^{-1})*	Exposure time (h)	Tested at 20° C†			Tested at 25° C†		
		Dead	Jaw locked	Surviving	Dead	Jaw locked	Surviving
1	1	1	1	37	2	17	0
	3	2	18	0	17	0	0
2	1	0	0	20	1	16	0
	3	0	9	10	12	6	0
	6	5	14	1	19	0	0
2·5	1	—	—	—	0	17	5
	3	0	1	19	10	16	0
3·5	1	—	—	—	0	1	20
	3	0	0	20	1	1	20

*Nominal concentration ±0·1 mg l^{-1}
†Nominal temperature ±0·2° C.

Small bubbles of gas appeared in the vicinity of the swim bladder anlage in some larvae around the 10th day, but the bladder did not become well inflated until the 11th or 12th day at the earliest. The recovery of resistance on the 11th and 12th days suggests that the neutral buoyancy imparted by the inflated swim bladder was involved. This possibility is supported by another observation. Some of the larvae transported from Two Island Lake on their first day after hatching did not develop inflated swim bladders, even after several weeks. These fish fed and grew like the rest, and the branchial region looked well developed, but they were not buoyant and had to keep swimming vigorously to stay above the substrate. When such fish were exposed to 1 mg O_2 l^{-1} all died, whereas none of those with fully inflated swim bladders was killed.

EFFECTS OF VARYING CONCENTRATION, TIME AND TEMPERATURE

Larvae from the 1975 collection were tested at four oxygen concentrations and three exposure times at 20° C and 25° C. They were tested at 1 and 2 mg O_2 l^{-1} on the fifth day after hatching, and at 2·5 and 3·5 mg O_2 l^{-1} on the sixth day. Survival increased as the oxygen concentration increased and decreased as the exposure time increased (Table II). At 20° C no larvae survived 3 h at 1 mg O_2 l^{-1}, half survived at 2 mg l^{-1}, most survived at 2·5 mg l^{-1} and all survived at 3·5 mg l^{-1}. Raising the temperature by 5° C increased the sensitivity to oxygen deficiency. At 25° C no larvae survived at 1 or 2 mg O_2 l^{-1} and few survived for even an hour at 2·5 mg, but nearly all survived at 3 h at 3·5 mg l^{-1}. All controls at around 8 mg l^{-1} survived at 25° C.

LARVAL BEHAVIOUR AT REDUCED OXYGEN CONCENTRATION

The results of tests conducted in 1977 and 1979 were so similar that they can be combined. Smallmouth bass larvae showed essentially the same abnormal activities at reduced oxygen concentrations as those described for largemouth larvae (Spoor, 1977). In air-saturated water the smallmouth larvae moved little, not

more than an occasional twitch, until the seventh day after hatching when a few began making short excursions a few centimetres above the substrate. But even on the first day after hatching, when the yolk made up about 60% of the total weight, the larvae became extremely agitated as soon as the oxygen concentration was lowered to 3 mg l^{-1}. The twitching movements increased to 30–50 min^{-1} and the larvae swam in lateral loops across the substrate and in vertical loops several centimetres above it. This activity subsided quickly when the flow of air-saturated water was restored or when the oxygen-deficient water was re-aerated. Controls in adjacent trays showed no change from their normal quiescence.

The larvae continued to respond in this manner until they were ready to swim up, apparently becoming even more sensitive as they grew. On the first day none was seen making vertical loops as far as the surface at 3 mg O_2 l^{-1}; on the second day one was seen; on the third day and subsequently surfacing was observed regularly at 3 and 3·5 mg O_2 l^{-1} and occasionally at 4 mg l^{-1}. Even 5 mg l^{-1} caused increased twitching and an occasional loop. By the time they swam up on the ninth or tenth day they showed no clear-cut change in activity at 4 mg l^{-1}, but continued to surface at 3 mg l^{-1}. By the 12th day 3 mg l^{-1} had no apparent effect on activity.

In addition to becoming more active, the larvae, which were normally black by the third or fourth day after hatching, became pale as the oxygen concentration was lowered, and dark again when air-saturated water was restored. This reaction outlasted the change in activity at low oxygen concentration. On the 12th day after hatching, when 3 mg O_2 l^{-1} no longer caused a noticeable increase in activity, the fish continued to turn pale, indicating that they were still sensitive to oxygen deficiency.

The behavioural response was affected by temperature. At 4 ml O_2 l^{-1} 8-day-old larvae were more active at 19·5° C than at 16·5° C, the activity at 19·5° C subsiding when the oxygen concentration was raised. Further, 12-day-old larvae that had become pale at 3 mg O_2 l^{-1} and 20° C became dark again when the temperature was lowered to about 17·5° C, and pale once more when warmed back to 20° C.

LARGEMOUTH AND SMALLMOUTH BASS LARVAL REQUIREMENTS COMPARED

The mean yolk-free weight of largemouth bass at 20° C was about 0·9 mg on the first day after hatching and 1·8 mg at swim-up on the fifth day; the mean rate of oxygen consumption for the larval period was approximately 0·03 μg $larva^{-1}$ min^{-1} (Spoor, 1977). The corresponding values for smallmouth larvae were 2·3 mg on the first day after hatching, 8·4 mg at swim-up on the ninth day, and a mean rate of oxygen consumption of approximately 0·07 μg $larva^{-1}$ min^{-1} (Table I). The smallmouth bass larva's greater oxygen requirement is consistent with its greater sensitivity to oxygen deficiency. The 8-day period during which the smallmouth larvae were killed within 3 h at 3 mg O_2 l^{-1} is in marked contrast to the largemouth's 1 day of extreme sensitivity to these conditions. The greater oxygen requirement and sensitivity, together with the longer period between hatching and swim-up, suggest that the smallmouth bass is considerably more susceptible than the largemouth to environmental deterioration and the burden of predation during development. This helps account for differences in habitat selection:

the smallmouth bass is considered less tolerant than the largemouth in its environmental requirements (Forbes & Richardson, 1920; Robbins & MacCrimmon, 1974).

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